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Mapping and characterization of neutralizing epitopes of glycoproteins gIII and gp50 of the Indiana-Funkhauser strain of pseudorabies virus

Brief Report

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Summary. Neutralizing monoclonal antibodies directed against pseudorabies glycoproteins gIII or gp 50 were produced. Using these antibodies in competitive binding ELISAs, three overlapping epitopes for gp 50 and two nonoverlapping epitopes for gIII were mapped.

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Pseudorabies (PR), a herpesvirus disease of swine, is a major cause of reproductive failure and neonatal mortality in susceptible animals [2]. Pseudorabies glycoproteins gIII and gp 50 have been identified as targets of neutralizing antibodies elicited during the immune response to the virus [1, 13]. Previous studies have shown that monoclonal antibodies directed against gIII and gp 50 provide passive protection from clinical disease in mice and swine [1, 4, 13]. Glycoprotein gp 50 has been suggested as a subunit vaccine candidate [4].

We further characterized the antigenic structures of PRV glycoproteins gIII and gp 50. A panel of monoclonal antibodies reactive against gIII and gp 50 were produced and characterized. Through the use of competitive binding enzyme-linked immunosorbent assays (ELISA), we were able to demonstrate multiple neutralizing epitopes for both of these viral proteins.

The Indiana-Funkhauser strain of PRV was used. For monoclonal antibody fusion inoculum, virus-infected fetal mouse fibroblasts of Balb/c origin were virus inactivated with acetylethyleneimine [6] and resuspended in phosphate buffered saline (PBS), pH 7.2. For all other assays infectious virus was harvested in cell supernatant after replication in porcine kidney (PK-15) cells.

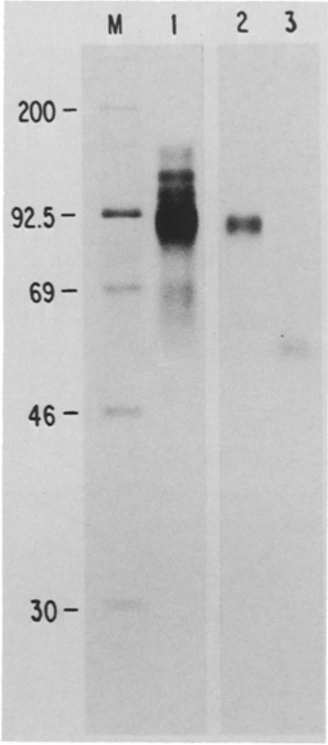

Fig. 1. Autoradiogram of pseudorabies viral glycoproteins immunoprecipitated with porcine polyclonal antiserum (*1*), monoclonal antibody against gIII (*2*), and monoclonal antibody against gp50 (*3*). *M* Marker

Monoclonal antibodies against pseudorabies virus were prepared by the fusion of spleen and sp2/0 myeloma cells as described by Van Deusen and Whetstone [10]. Three weeks prior to fusion, Balb/c mice were injected IP with 200 µl of the infected cell suspension (approximately 5×10^5 cells). A 200 µl IV booster was given 18 days later.

Clones were screened and characterized by a combination of radioimmunoprecipitation and virus neutralization assays. Radioimmunoprecipitation of PRV-infected cell lysates labeled with ^{14}C-glucosamine (30,000 cpm/sample) was performed as described elsewhere [7]. Precipitates were electrophoresed on 10% polyacrylamide gels and exposed to Kodak X-OMAT AR film. Monoclonal antibodies directed against PRV glycoproteins gIII and gp50 were selected for further study; a representative autoradiogram is shown in Fig. 1. The lower molecular weight band seen in the lane containing gIII represents an immature form of the protein as demonstrated by Ryan et al. [10].

Table 1 shows the results of the virus neutralization assays for monoclonal antibodies used in this study. The assays were performed as described by Wathen et al. [13], except that PK-15 cells were used and all antibodies were adjusted to a 1 mg/ml starting concentration. It is coincidental that all of the monoclonal antibodies that were isolated against PRV glycoprotein gIII or gp50 had neutralizing activity. All neutralized virus much more efficiently (16- to 512-fold) in the presence of complement although four out of nine had limited neutralizing activity without complement.

Table 1. Virus neutralization titers of monoclonal antibodies produced against pseudorabies virus glycoproteins gIII and gp50

Monoclonal antibody ID	Target protein	Titer with complement[a]	Titer without complement
F2E3.1E8	gIII	1:1,024[b]	<1:2[c]
3E3.1C4	gIII	1:512	<1:2
F4H6.1H3	gIII	1:512	<1:2
DPgIII	gIII	1:512	<1:2
F2A12.1C9	gp50	1:1,024	1:2
S4B1.1C11	gp50	1:512	1:4
S3G12.1B1	gp50	1:256	1:8
F3A9.1E4	gp50	1:256	<1:2
J2A3.1A4	gp50	1:64	1:4

[a] Each antibody was adjusted to 1 mg/ml before 2-fold dilutions were made. In complement-dependent assays, fresh rabbit serum was added to the virus antibody mixtures to give a final concentration of 3%

[b] Titers expressed as greatest antibody dilution protecting at least 50% of replicate samples from cytopathic effect

[c] No neutralization observed at dilutions used

Because of interference from extraneous components in raw ascites, monoclonal antibodies selected for mapping experiments were purified prior to their use. Purified unlabeled antibodies were prepared by protein A affinity chromatography according to manufacturer's instructions (Bio-Rad).

Antibody to be labeled was crudely fractionated from ascites with 50% saturated ammonium sulfate. Conjugation to horseradish peroxidase was accomplished by the periodate method [14]. No further purification was found to be necessary.

Plates used for the competitive binding ELISA were prepared essentially as described by Voller et al. [13]. Each well contained 10^6 pfu of virus or a similar dilution of uninfected PK-15 cell culture supernatant as a mock antigen. The plates were blocked with PBS + 1% skim milk.

Binding curves were established for each monoclonal antibody using serial dilutions of antibody in an indirect ELISA as described elsewhere [12]. Peroxidase-labeled goat anti-mouse conjugate (Kirkegaard-Perry, Gaithersburg, MD) was detected by an ABTS substrate solution [10 ml 12.5 mM citric acid (pH 3.6), 50 µl 40 mM 2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid), 80 µl 3.75% H_2O_2]. After a 30 min incubation, % absorbance was read at 405 nm/630 nm. Except for the addition of the goat anti-mouse conjugate, similar determinations were made for conjugated monoclonal antibodies. From these curves, working dilutions were calculated and made in Tween 20 wash buffer + 1% skim milk. Unlabeled antibody was used at a saturating concentration. Conjugated antibody was used at a level corresponding to the linear segment of the binding curve.

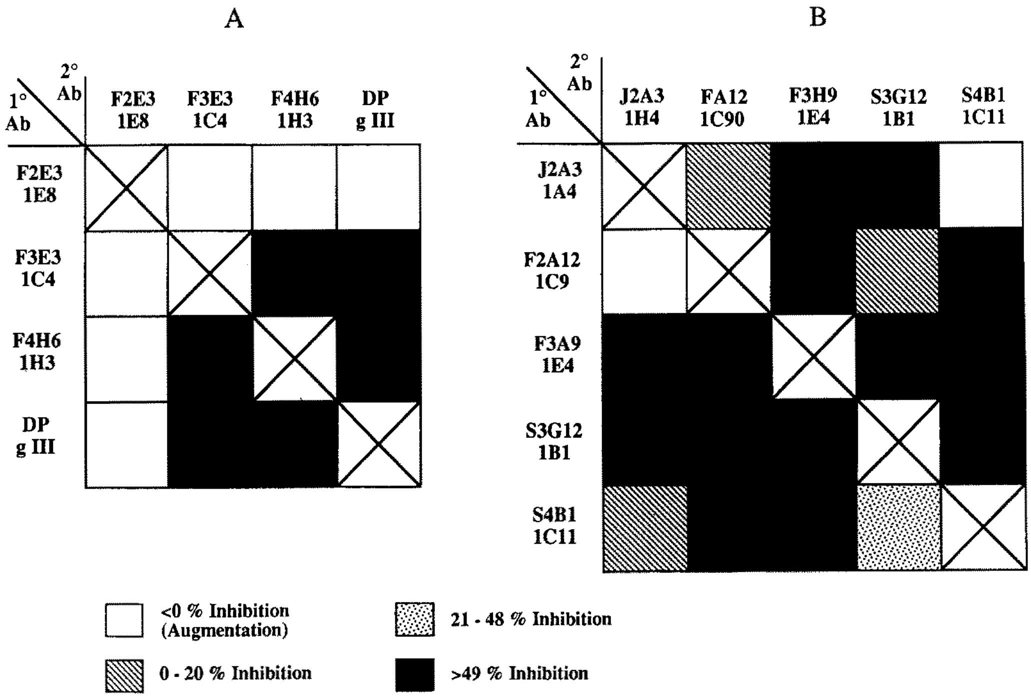

Fig. 2. Results of competitive binding ELISA for pairs of monoclonal antibodies against pseudorabies glycoproteins. **A** Antibodies against gIII. **B** Antibodies against gp50

For each binding competition, unlabeled primary antibody was allowed to incubate in plates for one hour. Without rinsing, an equal volume of peroxidase-labeled secondary antibody was added and incubated an additional hour. Rinsing and substrate development were performed as described above.

Each competition was completed in reciprocal combination to account for differences in monoclonal antibody avidity. Background absorbance was determined by incubation of antibodies in wells containing mock antigen, and this value was subtracted from total absorbance readings. Each secondary antibody was incubated without competing antibody to determine theoretical maximum binding levels. Percent inhibition or enhancement due to the competing antibody was calculated from average corrected absorbance readings using the formula described by Lussenhop et al. [3]:

[(%$A_{405/630}$ with competing antibody) − (%$A_{405-630}$ without competing antibody)] × 100/(%$A_{405/630}$ without competing antibody).

The results of our competition experiments are summarized in Fig. 2A, B. In Fig. 2A, three of the monoclonal antibodies directed against PRV glycoprotein gIII (DPgIII, F3E3.1C4, F4H6.1H3) were mutually inhibitory. Enhanced antibody binding was observed when the fourth antibody, F2E3.1E8, was present in a combination. This enhancement could have reflected a conformational change in the protein after binding by the primary antibody wherein the second epitope could have been rendered more assessable to binding.

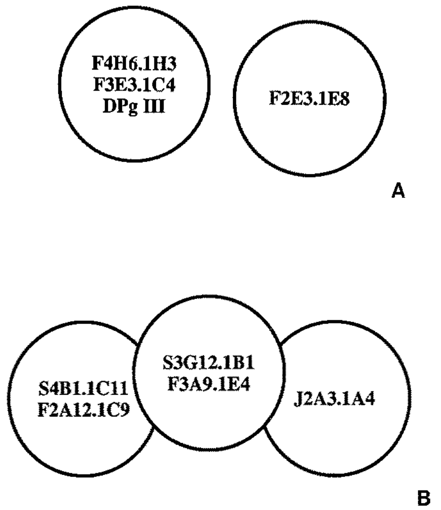

Fig. 3. Proposed topographical maps of neutralizing epitopes on pseudorabies glycoproteins mapped by the monoclonal antibodies indicated. **A** Glycoprotein gIII contains two nonoverlapping epitopes. **B** Glycoprotein gp 50 contains three overlapping epitopes

In Fig. 2B, four of the monoclonal antibodies directed against gp50 (S4B1.1C11, S3G12.1B1, F2A12.1C9, F3A9.1E4) were mutually inhibitory. When paired with monoclonal antibody J2A3.1A4, though, S3G12.1B1 and F3A9.1E4 were inhibitory but S4B1.1C11 and F2A12.1C9 were not.

Suggested topographical maps of the epitopes are illustrated in Fig. 3A, B. Viral glycoprotein gIII is represented by two nonoverlapping epitopes, one defined by the three mutually inhibitory antibodies and the other by the fourth antibody (Fig. 3A). Antibody binding at one of these epitopes does not appear to hinder binding at the other.

Glycoprotein gp50 is represented by three overlapping epitopes (Fig. 3B). Steric forces may inhibit simultaneous binding at adjacent epitopes but not at nonadjacent epitopes. The collinearity of epitopes on this topographical map does not necessarily imply sequence collinearity on the protein itself. The epitopes may share a functional spatial relationship by virtue of higher protein structure. Also, depicted single epitopes may actually be clusters of closely related epitopes that are inseparable on the basis of this assay.

Deletion mutant and subunit pseudorabies vaccines are currently being developed and marketed [8]. These products have an advantage over more traditional vaccines in that they allow discrimination between vaccinated and infected animals. Analysis of neutralizing epitopes is an important consideration in the development of such vaccines as only a part of the viral genome is presented to the animal. Vaccinates mounting a prompt, potent neutralizing response to virus challenge experience decreased severity and duration of clinical disease [5]. In this study, we have shown that at least two neutralizing epitopes appear to exist for PRV glycoprotein gIII while at least three neutralizing epitopes appear to exist for gp50. The relationships between these epitopes may be linear or functional. To this end, further characterization could be accomplished by Western blot analysis and ELISA studies using denatured viral proteins.

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